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Protective effect of phenolic extract of *Cyperus rotundus* rhizomes on myocardial infarction induced by isoproterenol in female rats

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Abstract Objective: To asses the protective effect of phenolic extract of *cyperus rotundus* rhizomes on biochemical in isoproterenol induced myocardial infraction. Methods: Myocardial infarction was induced in rats by intraperitoneally injection of isoproterenol (85mg/kg) for two consecutive days at an interval of 24 h. Rats were treated with phenolic extract of *cyperus rotundus* rhizomes at two doses (15mg/kg, 30 mg/kg) for period 21 days and isoproterenol was injected on the 21 th and 22 th day. At the end of experiment i.e. on the 23 th day biochemical changes were monitored from control and experimental groups. Results: ISO injected rats showed a significant increase in d-dimer levels. In addition, it also exhibited alteration in the levels of electrolytes (Na^+ and Cl^-). It also showed significant decrease in level of K^+. Pretreatment with phenolic extract of *cyperus rotundus* rhizomes significantly prevented the ISO induced alteration in biochemical changes. Conclusions: The present result shows that treatment with phenolic extract of *cyperus rotundus* rhizomes in ISO injected rats significantly attenuates induced myocardial infarction.

Keywords: Myocardial infraction, *cyperus rotundus*, Isopreterenol, electrolytes.

1. Introduction

A myocardial infarction occurs when an atherosclerotic plaque slowly builds up in the inner lining of a coronary artery and then suddenly ruptures, causing catastrophic thrombus formation, totally occluding the artery and preventing blood flow downstream. Cardiac hypertrophy is a general term signifying an increased workload and is characterized with an increase in cardiac mass in response to applied stimulus [1]. Prolongation of this process leads to congestive heart failure [HF] defined as a progressive syndrome that appears as the final phase of most cardiac diseases [2]. Myocardial infarction [MI] is manifested with the impaired systolic and diastolic function, ventricular dilatation and ultimately with congestive HF[3]. Numerous nonspecific manifestations may be recognized in patients with acute MI. Although they are not generally employed in establishing the diagnosis, awareness of their coexistence with infarction is important to avoid misinterpretation or erroneous diagnosis of other disorders [4].

The use of herbal medicines has been steadily increasing over the past decade to cure some of the disorders in human. Epidemiologists in India and international agencies such as the World Health Organization [WHO] have been sounding an alarm on the rapidly rising burden of CVD for the past 15 years. The reported prevalence of coronary heart disease [CHD] in adult surveys has risen four-fold in 40 years and even in rural areas the prevalence has doubled over the past 30 years [5].

Isoproterenol (ISO) is a synthetic catecholamine and beta-adrenergic agonist, which causes severe stress in the myocardium, resulting in infarct like necrosis of the heart muscle [6]. It has been reported that ISO generates free radicals and stimulates lipid peroxidation, which is a causative factor for irreversible damage to the myocardial membrane [7].

A considerable number of these plants/plant based products have been widely used. Therefore, interest in the examination of plants as potential sources of new drugs is increasing. In India, medicinal plants are traditionally used in the treatment of cardiovascular disease, as they are inexpensive, efficacious and safe [8]. Phenolic compounds exhibit multiple pharmacological properties such as anti-microbal, anti- allergenic and antioxidant [9].

Cyperus rotundus (cypearaceae), commonly known as nagarmotha shows antihyperlipidemic activity. The rhizomes of *Cyperus rotundus* on preliminary chemical analysis is found to contain flavonoids, β-sitosterol, sesquiterpenoids [4].The study is an effort in the same direction thus the present investigation was undertaken to evaluate the protective effect of phenolic extract of *cyperus rotundus* rhizomes on isoproterenol induced myocardial infarction in female rats.

2. Materials and Methods

2.1 *Drugs and Chemicals*

Isoproterenol was purchased from Cayman (British), hexane, acetic acid; sodium chloride and n-propanol were purchased from BDH (England).

2.2 *Plant Material*

Samples of *Cyperus rotundus* rhizomes were collected from local market of Nasiriyia city, Thi-qar, Iraq. It cleaned after that broke and grinded it by using electric grinder.

(500 g) of plant powdered material was defatted by washing five times with n-hexane (1L) at (60°C), then it was mixed with (800mL) of acetic acid (2% v/v), the mixture was placed in conical flask volume (2000mL) and put in water bath (60°C) for 8 hrs, then the extraction process done by reflex condenser. The mixture was heated at 50°C (water bath) for 15 min and left to cool. The suspension was filtered by Buchner funnel by Whitman No.1 filter paper and by the use of vacuum pump. The precipitate was canceled and the filtrate volume was measured. n-propanol was added in to filtrate with the same volume of filtrate. Then (NaCl) was added until it become solution super saturated. Then, it was evaporator by using rotary evaporator until drying [10].

2.3 *Experimental Protocol*

Sixty healthy adult female rats (*Rattus norvegicus*) weighing (190-200 g) of 9-10 weeks old were used in the present study. Animals were housed in the animal house of Biology Department, Science College, Thi-qar University, Iraq. Experimental animals were divided into ten groups (6 rats in each group) . upon the following designed.

Group-1: a control group; treated orally with distill water for 21 days .

Group-2: ISO (Isoproterenol hydrochloride) group; were injected I.P. with (85mg/kg, body weight) of ISO, twice an interval of 24 h , i.e ., on 22 th and 23 th day).

Group-3: treated orally with 15 mg /kg of phenolic extract of *C. rotundus* rhizomes once daily for 21 days .

Group-4: treated orally with 30 mg /Kg of phenolic extract of *C. rotundus* rhizomes once daily for 21 days .

Group-5: pretreated orally with (15mg/kg) of phenolic extract of *C. rotundus* rhizomes. once daily for 21 days, then injected I.P. with (85mg/kg) of ISO, twice an interval of 24 h, i.e., on 22 th and 23 th day).

Group-6: pretreated orally with (30mg/kg) of phenolic extract of C. *rotundus rhizomes*. once daily for 21 days, then injected I.P. with (85mg/kg) of ISO, twice an interval of 24 h, i.e., on 22 th and 23 th day).

2.4.Biochemical Estimation in Serum

5mL of blood were drawn from each animal of experimental groups, the sample was transferred into clean tube, left at room temperature for 15 minutes for clotting, centrifuged at 3000 rpm for 15 minutes, and then serum was separated and kept in a clean tube in the refrigerator at (-20 °C) until the time of assay. The serum was used for the estimation d-dimer. It was measured according to the method of the competitive ELISA kit, the used reagents were supplied by (My biosource, USA),

Sodium and Potassium were measured with commercial kits , the used reagents were supplied by (Randox, UK) and chloride was measured with commercial kit, the used reagents was supplied by (Biolabo, France).

2.5.Statistical Analysis

Statistical analysis was done using the software **SPSS** version 15.0;The results were expressed as mean ± standard deviations (mean ± SD) and LSD. Two way ANOVA-test was used to compare parameters in different studied groups. P-values($P \leq 0.05$) were considered statistically significant .

3. Results

The results showed a significant increase ($p \leq 0.05$) in the concentration of serum d-dimer, sodium and chloride in group (2) in comparison with group (1). There was non-significant difference in the concentration of serum D-dimer, sodium and chloride in groups (3 and 4) in comparison with group(1) and between them. Also, there was a significant decrease ($p \leq 0.05$) in the concentration of serum d-dimer, sodium and chloride in groups(5 and 6) in comparison with group (2). While there was non- significant differences in the concentration of serum d-dimer, sodium and chloride between groups (5 and 6). Also, there was a significant decrease ($p \leq 0.05$) in the concentration of serum potassium in group (2) in comparison with group(1).Also, there was non-significant differences in the concentration of serum potassium in groups (3,4 and 6) in comparison with group (1) and between them. While there was non- significant differences in the concentration of serum potassium between groups(2 and 5). While there was a significant increase ($p \leq 0.05$)in the concentration of serum potassium in group (6) in comparison with group (2) . Table (1).

Table 1. Serum Biochemical analysis

	D-dimer (µg/mL)	Na+(mmol/L)	Cl-(mmol/L)	K+(mmol/L)
Group(1)	0.50 ± 0.15 ^{c}	95.00± 6.82 ^{c}	80.60±2.85^{c}	8.97± 0.54 ^{a}
Group(2)	3.50±0.70 ^{a}	140.20±4.45^{a}	119.00±12.01^{a}	5.96± 0.81 ^{b}
Group(3)	0.45 ±0.15^{c}	99.10±1.50 ^{c}	82.50±1.53 ^{c}	8.96± 0.11^{a}
Group(4)	0.28±0.05 ^{b}	97.10±3.30 ^{c}	83.20±3.44^{c}	8.55±0.84 ^{a}
Group(5)	2.15±0.07 ^{b}	120.42± 4.08 ^{b}	94.45±1.80 ^{b}	6.05± 0.50 ^{b}
Group(6)	1.96±0.26 ^{b}	114.05±5.14 ^{b}	92.25±2.70 ^{b}	7.50± 0.68 ^{b}
LSD	1.30	7.83	7.15	1.06

* Each value represents mean ± SD values with non identical superscript (a , b or c …etc.) were considered significantly differences ($P \leq 0.05$).

4. Discussion

D-dimer is an enzymatic degradation product that forms as a result of plasmin lysis of cross-linked fibrin clots. The plasma level of d-dimer is important for assessing the patient's fibrinolytic status [11]. D-dimer levels rise because d-dimer is involved at an earlier stage in the pathophysiological process of MI [12]. In the present study, the protective role of phenolic extract of *cyperus rotundus*

rhizomes in ISO-induced MI was studied by decreasing the levels of d-dimer related to MI and improving the pathological changes of heart. Previous researchers have demonstrated that MI has proven to be associated with oxidative stress [13], apoptosis [14], and inflammation [15]. Therefore, the protective effect of phenolic extract of *cyperus rotundus* rhizomes on MI can be attributed to the following mechanisms.

In the cell, ATPases are closely associated with the plasma membrane and participate in the energy dependent transport of sodium, potassium, magnesium and calcium translocation. An increase in sodium along with decrease in potassium were observed in ISO injected rats which might be due to altered ATPases activity in membrane as a result of lipid peroxidation produced by ISO. Increased concentration of sodium might be due to decrease in Na+/K+ ATPase [16]. *Cyperus rotundus* rhizomes treatment can prevent the altered levels of electrolyte and these effects of *cyperus rotundus* rhizomes could be due to prevention of SH group of the ATPases from oxidative damage through the inhibition of peroxidation of membrane lipids indicating the membrane stabilizing effects of *cyperus rotundus rhizomes.*

5. Conclusion

The present study showed that the acute ISO injection in experimental animals induces MI which is confirmed by d-dimer levels. Pretreatment with phenolic extract of *cyperus rotundus* rhizomes prevents the ISO induced MI. These findings might by helpful to understand the beneficial effects of *cypers rotundus* rhizomes against ISO induced MI although further study is needed to confirm its mechanism.

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